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This article is available in The Thai Journal of Pharmaceutical Sciences: https://digital.car.chula.ac.th/tjps/vol46/iss5/9
Antidiabetic study and teratogenicity evaluation of ethanolic extract of Chromolaena odorata Linn.

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ABSTRACT

There is considerable interest in herbal medicine in developing antidiabetic alternative therapy based on plant-derived compounds that would reduce oral hypoglycemic agent-related adverse effects while staying safe during pregnancy. The purpose of this in vivo study was to evaluate the ethanolic extract of Chromolaena odorata L. leaves (ECOL) in alloxan-induced diabetes and its teratogenic effect. The hypoglycemic impact of ECOL increased when the extract dose was raised. Three doses of ECOL (125, 250, and 500 mg/kg body weight) showed a 27.29%, 34.68%, and 52.24% hypoglycemic effect, respectively. While, the standard insulin (1 IU/kg BW) group shown a 31.38% hypoglycemic effect ($p < 0.05$). The ECOL groups improved beta-cells in the islets of Langerhans in histological examinations of the pancreas. The ECOL groups showed no influence on fetal mortality, however, had a substantial impact on the fetal ossification process ($p < 0.05$) in the teratogenic study.

Keywords: ECOL, Alloxan, Fetal, Ossification

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by uncontrolled hyperglycemia in the blood. Uncontrolled hyperglycemia is also found in pregnant women called gestational diabetes mellitus (GDM). The global prevalence of GDM varies widely, from 1% to 28%, depending on population characteristics, screening methods, diagnostic criteria, and genetic factor.\(^\text{[1,2]}\) Nguyen et al.\(^\text{[3]}\) conclude that around 10.1% of pregnant women in Eastern and Southeastern Asia had GDM and the number of women with GDM varied substantially between and within countries. GDM has been associated with short-term and long-term adverse health outcomes for both mothers and their newborns.\(^\text{[4]}\) Risk factors that can accompany GDM conditions, including preeclampsia/eclampsia, complications during childbirth, and the risk of type 2 diabetes later in life (prevalence reaches 40–60%).\(^\text{[5]}\) While the risk factors in a newborn include macrosomia, shoulder dystocia, stillbirth, congenital abnormalities, premature birth, stunted fetal growth, hypoglycemia, hyperbilirubinemia, and hypocalcemia.\(^\text{[6]}\)

The most frequent medicine for treating diabetes is oral hypoglycemic agents (OHAs); however, most OHAs have been found to be unsafe during pregnancy and to have a number of side effects.\(^\text{[7,8]}\) As a result, there is a growing interest in herbal medicine to develop an alternative treatment made from plant-derived substances that will diminish OHAs-related adverse effects while remaining safe during pregnancy.\(^\text{[9]}\) The Komering tribe of South Sumatera, Indonesia, has traditionally used Chromolaena odorata L. leaves to treat diabetes mellitus. The scientific data for the ethanolic C. odorata L. leaves in terms of diabetic effectiveness and pregnancy safety are currently inadequate.

C. odorata L. has been reported to have antioxidant\(^\text{[10,11]}\) anti-inflammatory\(^\text{[12]}\), antimicrobe\(^\text{[13]}\), and antidiabetic\(^\text{[14-16]}\) properties. C. odorata L. leaves contain phenolic group, with major compounds are tamarixetin, trihydroxy monomethoxy flavanone, penta methoxy chalcone, eupatilin, p-coumaric acid, p-hydroxybenzoic acid, and protocatechuic acid.\(^\text{[17]}\) Tamarixetin is 4’-methyl ethers of quercetin. The total flavonoid content of C. odorata L. leaves ethanolic extract was 126.459 ± 0.163 mg/g expressed as quercetin.\(^\text{[11]}\) Quercetin has been known as a potent antioxidant, anti-inflammatory, and antidiabetic agent. Furthermore, protocatechuic acid is known to ameliorate streptozotocin-induced toxicity in
diabetic rats.[24] Ethanolic extract of C. odorata L. has LD₅₀ >5000 mg/kg BW.[25] The high LD₅₀ value obtained was a clear indication that the C. odorata L. could be safe for use. In subchronic toxicity study, aqueous extract of C. odorata L. relatively safe in dose under 538.5 mg/kg BW.[25] The goal of this study was to evaluate the ethanolic extract of C. odorata L. leaves in alloxan-induced diabetes and its teratogenic effect in the experimental animal.

**MATERIALS AND METHODS**

**Materials**

C. odorata L. leaves were selected for this study and were from South Sumatera, Indonesia. The plant has been identified at Andalas University Herbarium with certificate number 148/K-ID/ANDA/IV/2018. Preparation of the ethanolic leaves extract of C. odorata L. was based on Solihah et al.[11] The chemical used for this study was alloxan monohydrate (Sigma-Aldrich®), long-acting insulin gargle (Solostar®), glucose oxidase-peroxidase aminoantypyrine (GOD-PAP) kit (Diasys®), Hematoxylin-eosin stain, Alizarin-red stain, and all chemical reagents in analytical standard.

**In vitro Antioxidant Assay**

In this study, DPPH radical scavenging assay has been used to determine the in vitro antioxidant activity to allow rapid screening of substances. The scavenging ability of C. odorata L. leaves to DPPH radicals was measured according to a previously study.[21] A solution of 0.1 mM DPPH was prepared in ethanol p.a then 1 mL of 0.1 mM DPPH (Ab) added to 1 mL solution of extract (50, 100, 200, 400, and 800 ppm). The mixtures (As) were incubated at room temperature for 20 min then their absorbance were obtained at 516 nm. The scavenging activity is expressed as percent was calculated by equation (1) and the half-maximum inhibitory concentration (IC₅₀) value was calculated from the regression of dose-percent inhibition relationship.

\[
\text{Scavenging activity(\%) = } \frac{Ab - As}{Ab} \times 100 \%
\]

**Experimental Preparation for the Antidiabetic Test**

**Animal preparation**

Healthy male Wistar rats, weighing 150–200 g and age of 8–10 weeks, were used and 7th days were acclimated at the laboratory facility. All animals were maintained with approved animal care operating procedures consistently by the health research review committee Sriwijaya University (certificate number 83/kepkrismhfkunris/2018). They were fed the standard pellet diet and water ad libitum. They were kept in a polypropylene cage at 25 ± 2°C in a 12h light-dark cycle.[16] All animals were maintained with approved animal care operating procedures consistent with ethical approval number 022012032 from the research ethics committee Ahmad Dahlan University.

**Teratogenic effect test**

Animals were randomly divided into four groups: Control group (Gcontrol), diabetic control group (Gdiabetic), a diabetic group with standard insulin treatment (Ginsuline) with dose 1 IU/kg body weight (BW) in NaCl 0.9% solution, and three ethanolic extracts of C. odorata L. leaves (ECOL) groups with a dose 125, 250, and 500 mg/kg BW in Na CMC 1% suspension, assigned as GECOL₁₂₅, GECOL₂₅₀, and GECOL₅₀₀ respectively. Each group consists of six rats.[24] All animals were measured the BW and fasting glucose level after 1 week acclimatized in laboratory condition (T0). Type 1 diabetes mellitus was induced in all groups, except Gcontrol by a single intraperitoneal administration of 130 mg/kg BW alloxan monohydrate dissolved in NaCl 0.9% on overnight fasted rats.[22] Three days after induction, the BW and fasting glucose level were remeasured (T1). Further tests were conducted on rats with blood glucose levels of more than 200mg/dL.[23] The treatments start on T2, Gcontrol and Gdiabetic were received Na CMC 1% solution orally, GECOL were received the suspension of ECOL by oral, while insulin by subcutaneous, daily for 20 days. The BW and fasting glucose level were measured every 5 days (assigned as T2, T3, T4, and T5). Animals were euthanized by cervical dislocation after treatment completion for immunohistochemical study.[24]

**Measurement of glucose level**

Fasting glucose level was measured by collecting blood samples from the retro-orbital plexus. Serum and blood cells were separated by centrifugation. Serum glucose level was measured using the GOD-PAP enzymatic colorimetric spectrophotometrically.[22]

**Immunohistochemical study**

To evaluate any changes in pancreatic beta-cells after treatment, the pancreas was harvested from euthanized rats. Pancreatic tissues in the group were collected in 10% neutral buffered formalin, and then it was processed and embedded in paraffin wax. Sections were cut using microtome with 5 μm of thickness and stained with hematoxylin-eosin. The sections were examined microscopically for the evaluation of histopathological changes.[25]

**Experimental Preparation for the Teratogenic Test**

**Animal preparation**

Healthy Swiss-Webster mice, weighing 28–34 g and ages of 8–10 weeks, were used for this study and were acclimated for 7 days in a laboratory facility. They are allowed to access standard pellet and water ad libitum for 7 days in a laboratory facility. They are allowed to access standard pellet and water ad libitum. They were kept in a polypropylene cage at 25 ± 2°C in a 12h light-dark cycle.[16] All animals were maintained with approved animal care operating procedures consistent with ethical approval number 022012032 from the research ethics committee Ahmad Dahlan University.
were examined for vaginal smears. If sperms were discovered in the vaginal smear, female mice were considered fertilized and assigned as the 0th gestation day.26

The treatment was given during the organogenesis period (9th–17th gestation day). Cesarean section was performed on pregnant mice on the 18th day to separate the fetuses. The number of live fetuses, bodyweight of fetuses, resorption, and morphological abnormalities was recorded.

Skeletal preparation
The skeletal preparation procedure was described before by Fajriaty et al.26 The fetus was fixed by ethanol for 1 week, then skinned and the internal organs were removed. Fetuses were fixed by potassium hydroxide 0.5% solution for <24 h and then by hydrogen peroxide 1% for 2–3 h. The fetus was soaked by aqua dest for 10 min and then immersed in alizarin red solution for <24 h. The fetus is gradually soaked in a solution of 5, 20, 40, and 80% glycerol for each week.

Data Analysis
IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA) was used for data processing and analysis. All data were expressed as mean ± standard deviation, and then was analyzed using one-way ANOVA followed by Duncan’s post hoc test. Data were evaluated with a 95% confidence level (P < 0.05). Histopathological data were analyzed descriptively.

RESULTS AND DISCUSSION
Antioxidant of Extract
Free radicals react with proteins, lipids, brain cells, collagen, connective tissue, blood vessels, immune cells, and DNA, among other things. If left uncontrolled, free-radical reactions cause oxidative stress, which can lead to increased susceptibility to illness, premature aging, heart disease, chronic inflammations in a range of organs and tissues, arthritis, asthma, diabetes, and stroke. DPPH scavenging is a widely used method to evaluate antioxidant activities in a relatively short time when compared with other methods. This test is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity of specific compounds or extracts. Figure 1 exhibits the DPPH radical scavenging activity of C. odorata L. ethanolic leaves extract. Antioxidant activity is expressed by the value of the scavenging activity of DPPH. The concentration of extract and scavenging activity of DPPH has a linear relationship, the highest concentration of extract has the highest scavenging activity of DPPH. The C. odorata L. ethanolic leaves extract 800 ppm has 76.454 ± 0.683% scavenging activity of DPPH.

The half-maximum inhibitory concentration (IC$_{50}$) value was calculated from the regression of the concentration-scavenging activity relationship. The IC$_{50}$ value of C. odorata L. ethanolic extract is 84.319 ppm. The extract has strong antioxidant activity (IC$_{50}$ = 50–100 ppm). Polyphenols, or phenolic compounds, are now a prominent focus of research since they are thought to be effective chain-breaking antioxidants, as well as anti-inflammatory, antibacterial, antiviral, and anticancer agents. Total flavonoid compound levels in C. odorata L. leaves ethanolic extract were found to be 126.459 mgQE/g extract.\textsuperscript{[11]} The extract’s high flavonoid concentration has directly contributed to its antioxidant action by neutralizing free radicals. The current study’s findings clearly show that polyphenols are key elements of this plant in terms of antioxidant capacity.

Antidiabetic Evaluation
Alloxan-induced diabetes
Baseline serum glucose level was determined before alloxan injection (T0). All rats showed normal serum glucose levels with no significant difference among the groups [Table 1 and Figure 2]. The successful diabetic induction with alloxan monohydrate intraperitoneally was marked by a sudden increase in serum glucose level at >200 mg/dL. Three days after alloxan injection (T1), all alloxan-induced groups (G$_{\text{diabetes}}$, G$_{\text{insulin}}$, G$_{\text{ECOL125}}$, G$_{\text{ECOL250}}$, and G$_{\text{ECOL500}}$) have serum glucose level >200 mg/dL [Table 1 and Figure 2]. No visible signs of toxicity such as convulsions, restlessness, excitement, respiratory distress, or coma were observed. All animals remained alive during 20 days of observation.

The effect of C. odorata L. leaves extract on BW
Alloxan is the main compound used to induce Type 1 diabetes mellitus models in animal studies that contribute to the functional defect of pancreatic beta-cells and then lead to hyperglycemia and weight loss.\textsuperscript{[23]} The Type 1 diabetic mellitus condition has induced the reduction of BW as the result of...
insulin deficiency that prevents the utilization of glucose for energy sources and induces the consumption of stored fats and muscle protein for energy. After alloxan injection, all groups were significantly reduced of BW at T1 (P < 0.05), except the control group [Figure 3].

After treatment at first 5 days (T2), all groups saw an increase in BW with time, except for the diabetic group. At T3, differences between groups began to emerge (P < 0.05). The weight of the control group, insulin group, and ECOL250 group all increased with the same trend at T3 (P > 0.05). While the ECOL125 and ECOL500 groups had the same increasing trend (P > 0.05) with insulin at T4. When compared to the ECOL125 and ECOL500 groups, the ECOL250 group gained the most weight (P < 0.05), even more than the insulin group (P > 0.05). This demonstrates that a dose of 250 mg/kg BW of C. odorata L. leaves ethanolic extract had the best action in preventing muscle wasting due to hyperglycemic status and improved metabolic activity. According to Omonije et al. (2019),[15] a dosage of 600 mg/kg BW of the methanolic extract of C. odorata L. showed the highest capacity to raise the weight of alloxan-induced diabetic rats. The difference effect between C. odorata L. leaf and root extracts is related to their phytochemical composition. Tannins, alkaloids, flavonoids, terpenoids, saponins, and phenolic acids are more abundant in the leaves of C. odorata L. than in the roots (P < 0.05).[27]

Table 1: The serum glucose level (mg/dL) of rats before and after induction with Alloxan monohydrate 130 mg/Kg body weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum glucose level (mg/dL)</th>
<th>Before induction (mean±SD)</th>
<th>After induction (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>116.21±8.53</td>
<td>120.36±4.66</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td>115.00±4.33</td>
<td>238.84±7.26***</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>117.27±6.99</td>
<td>239.38±6.04***</td>
</tr>
<tr>
<td>ECOL125</td>
<td></td>
<td>95.14±16.89</td>
<td>249.54±11.70***</td>
</tr>
<tr>
<td>ECOL250</td>
<td></td>
<td>88.01±5.49</td>
<td>257.15±14.61***</td>
</tr>
<tr>
<td>ECOL500</td>
<td></td>
<td>110.28±18.71</td>
<td>217.72±10.83***</td>
</tr>
</tbody>
</table>

Table 1: The serum glucose level (mg/dL) of rats before and after induction with Alloxan monohydrate 130 mg/Kg body weight

The effect of C. odorata L. leaves extract on serum glucose level

The diabetes-induced group had a serum glucose level of >200 mg/dL, which was considerably higher than the serum glucose level of the control group (P < 0.0001). The decrease in serum glucose levels began at T2, and rats in the ECOL500 group had a significantly lower serum glucose level than the other groups (P < 0.0001). The decrease in serum glucose levels in the treatment group was significantly different from the diabetes group on T3-T5 (P < 0.05). Meanwhile, on T4-T5, the C. odorata L. leaves ethanolic extract treatment group showed a better decrease in serum glucose levels than insulin (P < 0.05).

Based on the data in Figure 2, the ECOL125, 250, and 500 groups had a decrease in serum glucose levels of 27.29%, 34.68%, and 52.24%, respectively. While the insulin group had a 31.38% decrease in serum glucose levels. The previous studies have found that C. odorata L. extract has hypoglycemic effects.[15,16,27-29] The presence of phenols, flavonoids, alkaloids, tannins, and saponins in the leaves of C. odorata L. has been observed to have a reducing effect on blood glucose.[14,16,30] The presence of these phytochemicals increases insulin synthesis in the Langerhans islet, resulting in a decrease in blood glucose levels. The hypoglycemic action of C. odorata L. could be explained by increased insulin production, inhibition of internal glucose synthesis, reduction of gut glucose absorption, and beta-cell regeneration, as these processes, have been shown to lower the glucose levels.[15,11] However, further research is needed to elucidate the most prominent mechanism.

**Immunohistochemical findings**

Figure 4 shows immunohistochemical investigations of the pancreatic. Langerhans islet structure (bold arrow) differs in the alloxan-induced group of rats compared to the control group. Induction of alloxan promotes beta-cell necrosis (thin arrow). Alloxan has a diabetic effect due to its rapid uptake by beta-cells and the generation of free radicals, which beta-cells have poor defense mechanisms against. Alloxan is reduced to dialuric acid and then oxidized back to alloxan, resulting in a redox cycle that generates superoxide radicals, which are disseminated to form hydrogen peroxide and subsequently extremely reactive hydroxyl radicals, which cause beta-cell DNA breakage.[32] Other methods of alloxan-induced beta-cell injury include the oxidation of critical-SH groups, particularly those of glucokinase,[33] and disruptions in intracellular calcium homeostasis.[34]

Based on Figure 4, the ECOL groups were shown improved beta-cells in the islets of Langerhans better than G_insulin groups. The insulin group showed shrinkage of the islets of Langerhans and there was beta-cell necrosis. The previous studies have been shown that C. odorata L. extract influenced the amount of beta-cell function restoration and could regenerate beta-cells to levels comparable to the normal control group.[16,35] The C. odorata L. ethanolic leaves extract has a strong antioxidant activity with an IC50 value of 84.319 ppm. Solihah et al. (2020)[11]
had reported that the ethanolic extract of C. odorata L. leaves exhibits high antioxidant potential by inhibiting serum MDA formation. The presence of phytochemicals in the ethanolic C. odorata L. leaves extract regulates free radicals, reducing oxidative stress induced by diabetes induction. The amount of phenolic and flavonoid content in the ethanolic extract of C. odorata L. leaves can inhibit enzymes involved in the formation of free radicals.\[36\]

**Teratogenic Evaluation**

**Estrus cycle**

The estrus cycle is a reproductive phase that occurs regularly in non-primate female animals and is distinguished by changes in physiology and behavior. In rats, the estrus cycle lasts 4–5 days and is divided into four phases: Proestrus, estrus, metestrus, and diestrus.\[37\] Estrus cycle in mice was observed using a light microscope with 400× magnification. The result showed that the mice’s estrus cycle was normal at 5 days [Figure 5].

In vaginal smear morphology, the estrus cycle has both nucleated cells and cornified cells.\[37\] Two female mice that were in the proestrus to estrus cycle were mated with one male mouse. After the mating procedure was completed the next day, the female mice’s vaginal swabs were examined again for the presence of sperm. The presence of sperm in a vaginal smear suggests successful mating and was identified as the day of gestation [Figure 6]. The treatment was given from the 9th until the 18th gestation day, during the organogenesis stage. The organogenesis stage is a vital period in the pregnancy, when fetus cells differentiate to become tissues and organs, during this period fetus is vulnerable to teratogenic drugs.

**The effect of C. odorata L. leaves on the morphology of fetus**

On the 18th day of pregnancy, a cesarean section was performed. This prevents cannibalism of mice that gives birth normally will tend to eat their defective heredity, so it can interfere with the observation of fetal abnormalities. Table 1 shows that all groups did not show any mortality of fetus, all fetuses were alive and implanted. The number of fetus lives in all groups is not significantly different (P > 0.05).

Based on Table 2 dan Figure 7, treatment with ethanolic extract of C. odorata L. leaves dose 250 mg/kg BW had 3.81% fetal resorption [Figure 7d] and 1.84% fetal hematoma [Figure 7b]. Treatment with the ethanolic extract of C. odorata L. leaves dose 500 mg/kg BW had 8.31% fetal resorption [Figure 7d] and 1.66% fetal hemorrhage [Figure 7c]. Fetal resorption, hematoma, and hemorrhage occur due to teratogenic effects. Resorption can be caused by morphological abnormalities with various body defects that end in death.\[26\] Hematoma and hemorrhage occur because there are phytochemical compounds that can penetrate the placental barrier, causing an osmotic imbalance and defects in the cardiovascular system. The administration of the ethanolic extract of C. odorata L. leaves was no significant differences in the incidence of resorption, hematoma, and hemorrhage in mouse fetuses (P > 0.05).
The effect of C. odorata L. leaves on BW fetus

The fetal BW is a fairly sensitive parameter on teratogenic effect. Based on Figure 8, C. odorata L. leaves did not affect the fetal BW ($P > 0.05$). The lightest effect of teratogenous compounds is the reduction of fetal BW. The reduction of fetal BW is one of the parameters for fetal growth inhibition due to disruption of growth-based processes such as cell division, cell interactions, metabolism, and biosynthetic reduction in cells.\[38\]

The effect of C. odorata L. leaves on skeletal formation

The observation of bone ossification is an excellent diagnostic for identifying the teratogenic properties of drugs and indicative of fetal growth retardation. Observations on the growth of fetal skeleton were performed using Alizarin red stain. The parameters of fetal skeletal abnormalities shown in Table 3 include the interparietal and supraoccipital skull bones, cervical vertebral bodies, Sacro caudal vertebral bodies, sternum (asymmetrical, cleaved, and dumbbell-shaped), metacarpal, and metatarsal limbs (proximal, intermediate, and distal phalanges).

Based on the data in Table 3, mice that were given ECOL had abnormalities of bone formation in the fetus. The mean of delayed intraparietal, supraoccipital, Sacro caudal, and dan cervical vertebrae bone ossification increased at the treatment dose compared to the control group ($P < 0.05$). Cervical vertebrae defects were shown in the formation of asymmetrical, cleaved, and dumbbell-shape of the sternum [Figure 9]. Each dosage of ethanolic extract C. odorata L. leaves has a different effect on the delayed ossification of a limb bone, either metacarpal or metatarsal.

The delay in bone ossification is considered to be related to cytotoxic compounds inhibiting osteoblast cell growth and inducing cell death. Teratogenic agents can interfere with cell death in variety of ways, including direct toxicity to cells, disturbance of the normal balance of mitogenic, homeostatic, and involution stimuli, and alteration of the sensitivity of the responsive cells to these or other signals. A prolonged phase of cell cycle arrest leads to abundant levels of cell death and embryotoxicity.\[39\]

Bone tissue is a dynamic entity that is highly responsive to both genetic and environmental factors. Environmentally induced placental damage also alters fetal skeletal formation. Appropriate development of the placenta is crucial to normal fetal development, and damage to placental trophoblast and labyrinthine endothelium is thought to adversely affect the fetus by diminished production of cytokines and growth factors, and diminished transplacental transport of nutrients.\[40\] To the best of our knowledge, this study is the first to report the teratogenic effects of C. odorata L. leaves. Plant phenolics are potent antioxidant free radical scavengers that protect cell viability by lowering DNA adduct formation, lipid peroxidation, protein carbonylation, and mitochondrial dysfunction. A phenolic compound in C. odorata L. leaves such as quercetin, tamarixetin, eupatilin, kaempferide, sinensetin, and rhamnetin are variously inducing cell death. The quantity of hydroxyl groups on the phenyl ring directly correlates to flavonoid potency. However, at low concentrations and in the presence of a free radical source, quercetin demonstrates prooxidant activity, which might aggravate rather than prevent against oxidative stress damage.\[41\] Another study

<table>
<thead>
<tr>
<th>Table 2: Fetal morphological abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameters</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Fetus live</td>
</tr>
<tr>
<td>Fetus dead</td>
</tr>
<tr>
<td>Implantation</td>
</tr>
<tr>
<td>Resorption</td>
</tr>
<tr>
<td>Hematoma</td>
</tr>
<tr>
<td>Hemorrhage</td>
</tr>
</tbody>
</table>

Figure 6: The presence of sperm in a mice vaginal smear

Figure 7: Morphology of fetus (a) normal fetus, (b) a fetus with hematoma, (c) a fetus with hemorrhage, (d) resorption fetus
discusses a quercetin paradox in which quercetin behaves as an antioxidant in reduced form, but reacts with protein thiols in oxidized form, producing adducts that trigger cytotoxicity and apoptosis.\[^{42}\] Rhamnetin induces apoptosis in cancer cell-line.\[^{43}\] Eupatilin induced the G2/M phase cell cycle arrest and apoptosis on malignant bone tumor.\[^{44}\] Eupatilin has no effects on osteoblast differentiation and shows cytotoxicity on osteoblast.\[^{45}\] Kaemferide induces accumulation of cells in the sub G0 phase, illustrating the induction of apoptosis.\[^{46}\] Tamarixetin inhibits cell proliferation, arrests the G2/M cell cycle, and induces apoptosis.\[^{47}\] Sinensetin induces cell cycle arrest in the G0/G1 phase.\[^{48}\] Our group is presently doing follow-up investigations to assess the degree of oxidative lipid peroxidation using placental malondialdehyde measurement.

Table 3: Skeletal formation and alterations in fetuses

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ECOL125</th>
<th>ECOL250</th>
<th>ECOL500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraparietal delay ossification</td>
<td>0(^a)</td>
<td>0.33±0.52(^{ab})</td>
<td>1.16±0.82(^{bc})</td>
<td>1.50±1.36(^c)</td>
</tr>
<tr>
<td>Supraoccipital delay ossification</td>
<td>0(^a)</td>
<td>0.67±0.82(^{ab})</td>
<td>1.50±1.05(^{bc})</td>
<td>2.00±0.63(^{c})</td>
</tr>
<tr>
<td>Sacro caudal bone</td>
<td>13.00±0(^a)</td>
<td>11.45±0.35(^{ab})</td>
<td>10.73±0.53(^{bc})</td>
<td>8.70±0.20(^{c})</td>
</tr>
<tr>
<td>Cervical vertebrae bone</td>
<td>7.00±0(^a)</td>
<td>6.54±0.35(^{bc})</td>
<td>2.07±0.56(^{cd})</td>
<td>1.12±0.15(^{d})</td>
</tr>
<tr>
<td>Asymetrical sternum shape</td>
<td>0</td>
<td>0.83±1.16</td>
<td>0.83±1.16</td>
<td>1.33±1.21</td>
</tr>
<tr>
<td>Sternum cleaved shape</td>
<td>0(^a)</td>
<td>1.83±0.40(^{ab})</td>
<td>1.50±0.83(^{bc})</td>
<td>0.33±0.51(^{ab})</td>
</tr>
<tr>
<td>Sternum dumbbell shape</td>
<td>0(^a)</td>
<td>1.00±0.63(^{ac})</td>
<td>1.42±1.16(^{abc})</td>
<td>0.5±0.83(^{ab})</td>
</tr>
<tr>
<td>Metacarpal (pp)</td>
<td>5.00±0(^a)</td>
<td>4.84±0.19(^{bc})</td>
<td>4.28±0.25(^{c})</td>
<td>4.70±0.18(^{b})</td>
</tr>
<tr>
<td>Metacarpal (ip)</td>
<td>4.00±0(^a)</td>
<td>3.97±0.04(^{c})</td>
<td>3.28±0.20(^{c})</td>
<td>3.70±0.13(^{c})</td>
</tr>
<tr>
<td>Metacarpal (dp)</td>
<td>5.00±0(^{ab})</td>
<td>4.87±0.19(^{bc})</td>
<td>4.13±0.28(^{c})</td>
<td>4.59±0.25(^{c})</td>
</tr>
<tr>
<td>Metatarsal (pp)</td>
<td>5.00±0(^{ab})</td>
<td>4.97±0.05(^{bc})</td>
<td>4.39±0.14(^{c})</td>
<td>4.80±0.06(^{bc})</td>
</tr>
<tr>
<td>Metatarsal (ip)</td>
<td>4.00±0(^{a})</td>
<td>3.81±0.32(^{bc})</td>
<td>2.94±0.36(^{c})</td>
<td>3.71±0.28(^{c})</td>
</tr>
<tr>
<td>Metatarsal (dp)</td>
<td>5.00±0(^{ab})</td>
<td>4.92±0.12(^{bc})</td>
<td>4.61±0.32(^{c})</td>
<td>4.84±0.22(^{bc})</td>
</tr>
</tbody>
</table>

pp: Proximal phalanges, ip: Intermediate phalanges, dp: Distal phalanges, numbers in the same row followed by the different superscript letters (\(^{a,b,c}\)) are significantly different (\(P<0.05\)).
to further describe placental oxidative stress and impaired fetal skeletal development.

CONCLUSIONS

C. odorata L. leaves have the potential to be developed as diabetic alternative medication. However, it should be avoided during pregnancy, especially during the organogenesis phase. Ethanolic extract of C. odorata L. leaves has a significant impact on the fetal ossification process.

ACKNOWLEDGMENT

The authors wish to acknowledge Sriwijaya University that supports this research through SATEKS grant contract no.0106.098/UN9/SB3.LP2M.PT/2021.

REFERENCES